Maryland Biological Stream Survey

Laboratory Methods for Benthic Macroinvertebrate Processing and Taxonomy

Maryland Department of Natural Resources
Monitoring and Non-tidal
Assessment Division
Ecological Assessment Program
580 Taylor Avenue, C-2
Annapolis, Maryland 21401

November 2000

CBWP-MANTA-EA-00-6

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Prepared by Dan Boward and Ellen Friedman

Maryland Department of Natural Resources
Monitoring and Non-tidal Assessment Division
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Contents

1 Introduction

This manual describes the methods used for benthic macroinvertebrate processing, identification, and data management for Maryland Department of Natural Resources Maryland Biological Stream Survey (MBSS). It is intended to serve as the Standard Operating Procedure for DNR staff involved in benthic processing and identification as well as a guide for other organizations wishing to use comparable methods. Field methods for MBSS benthic collecting may be found in Kazyak (2000) and Boward (2000).

2 Sample Check-In and Inspection

Upon arrival at the DNR Field Office (see Contact Information, Appendix A) list each sample on the Benthic Macroinvertebrate Sample Chain of Custody Form (Appendix B).

Each sample bucket is given a unique “Log Number” by Field Office staff. The Log Number is sequential for each calendar year with the first two digits indicating the year of collection and the last four digits indicating the sequential number of each sample bucket in the order that it is received by the lab (e.g., 000487 is sample bucket number 487 in calendar year 2000). Log Numbers, which are kept in a separate notebook for each calendar year, are especially useful in avoiding confusion related to duplicate samples taken at the same site.

Check each sample bucket for an adequate quantity of preservative (see Appendix C for a description of preservative). Ideally, there should be about twice as much preservative as there is sample material (by volume). If preservative is low due to spillage or evaporation, add more. Check sample buckets for cracks and poorly-fitting lids. Correct these problems as needed.

Store samples in an area with good ventilation (ambient air temperature should not exceed 100°F) until processed.

3 Laboratory

3.1 Preparation and Subsampling

Fill out all information at the top of a blank MBSS Benthic Macroinvertebrate Bench Sheet (Appendix D). Under a fume hood, remove the sample bucket lid and inspect the sample contents. Verify that sample numbers on both outside and inside labels are the same and complete. Note any instances of sample drying, dried organisms, mold, unusual color or odors, etc. in the comments section of the Bench Sheet.

Over a large and well-ventilated sink, pour the sample contents through a U.S. #30 (595μ mesh) sieve, catching the Synasol in a clean bucket positioned beneath the sieve. Once most of the Synasol is poured through the sieve, remove it from the sink. Gently rinse the interior of the sample bucket with tap water (sides, bottom, and lid) into the sieve until the Synasol odor is undetectable. Check that all organisms are removed from the sample bucket. Save the Synasol in the original labeled sample bucket for sortate storage (only Synasol that is not degraded [i.e., without a pungent odor or without discoloration] is saved for reuse). Rinse the sample material on the sieve with tap water to remove fine sediments. Clean large objects such as stones, sticks, and large leaves with a scrub brush to remove organisms. Discard these large objects after inspection.

Position the subsampling tray (see Appendix C for a description of MBSS standard subsampling tray) on a flat and level surface in good light. Place about ½ to 1 inch of tap water in the tray (or enough to completely cover sample material). Rinse the contents of the sieve into the subsampling tray. Use a squirt bottle to remove all sample material from the sieve. Spread the sample material evenly over the entire tray bottom. Allow sample material to hydrate for about 10 minutes. (Note: If the quantity of sample material is more than one sample bucket (about 86 ounces), the material may be split into (approximate) halves and steps x through x repeated for each half).

Using a random numbers table or a similar method of choosing numbers, randomly choose a number between 1 and 100 (there are 100 5cm grids in the subsampling tray). After positioning a Tensor lamp over the grid to be picked, begin removing organisms from the randomly chosen grid with forceps and place them in a watch glass containing preservative. It is advisable to sort major groups of organisms (e.g., to order plus family Chironomidae) into separate watch glasses upon removal from the subsampling tray. Keep a tally of the total number of organisms removed from the subsample tray.

If the total number of organisms removed from the first grid is equal to or greater than 120, subsampling is complete for the sample. If not, repeat the above process for another randomly-chosen grid. Continue this process until at least 120 organisms have been subsampled. The last grid chosen must be picked in it's entirety. For some samples, the total number of organisms may be less than 120, even after picking all grids in the subsample tray. Note: this process is normally referred to as "100 organism subsampling". The 120 organism target is used to allow for organisms that are missing parts needed for identification or non-organism material counted in the subsample.

Once the target number of organisms is tallied, note the number of grids required for the subsample on the Bench Sheet. If the sample was split and subsampled twice, make a note of the number of grids needed to get the first and second group of (approximately) 50 organisms. Make sure all watch glasses containing the subsampled organisms are securely covered and

properly labeled, preferably with separate labels for each watch glass, written in pencil. These labels should contain the Station ID, date collected and log number. If the organisms in the subsample will not be identified for several days, cover the watch glasses with parafilm in addition to the cover or transfer the organisms to an 80ml snap top vial.

After subsampling, pour the remaining sample material back into the laboratory sieve, allowing the water from the tray to go down the drain. Place the sample material back into the original sample bucket containing preservative and store as described in Section 6.0.

Make sure the subsample tray and laboratory sieve are rinsed well and free from remaining organisms prior to beginning another sample.

3.2 Identification

A list of taxonomic keys used for the identification of MBSS benthic macroinvertebrates can be found in Appendix E.

3.2.1 Core and Targeted MBSS

For the Random and Targeted MBSS samples, most organisms are identified to genus, if possible, using stereo scopes. Exceptions, and their corresponding target taxonomic level, include: Oligochaeta (family), Nematoda (phylum), Nematomorpha (family), etc. Those taxa not identifiable to genus (due to small size or damage) may be left at family or higher. These are noted on the bench sheet at the higher taxonomic level. Counts at levels higher than genus (except those noted above) are not assumed to be different from those identified to genus (see Data Entry below). Likewise, counts at levels higher than family are not assumed to be different from those identified to family.

3.2.1.1 The process for identifying Chironomid larvae is as follows:

General

Divide Chironomid larvae into Subfamily (i.e., Chironominae, Orthocladiinae, Tanypodinae, Diamesinae) or Tribe (i.e., Tanytarsini, Chronomini) and count the total number in each group. Identify (using slide mounts...see below) approximately 20% of the individual larvae within each Subfamily or Tribe. Once all 20% subsamples are identified, multiply the counts of all genera by five and record the total extrapolated number of genera for the entire Chironomid group. Note: if either the total number of Chironomids or the total number of individuals within a Subfamily or Tribe is ten or less, all larvae are identified (no subsampling is performed).

Clearing and mounting larvae

Remove Chironomid larvae from the Synasol and place in deionized water for about 10 minutes. Ensure that the larvae are totally immersed in the water and not floating. Place the larvae in 10% KOH in a small heat-resistant crucible. Heat them on low heat using a hotplate until the internal tissues are clear. Place the larvae in deionized water again for about 5 minutes. Return the larvae to Synasol.

With a drop or two of Synasol on a microscope slide, place several cleared larvae in a row with all heads toward one edge of the slide and dorsum down (mouthparts upward). Do not allow the larvae to dry, as air bubbles within the integument may block essential structures from view. Add one or two drops of mounting media (CMCP 10/CMCP 9AF) next to the larvae. Carefully lower a cover slip (one edge down first) over the larvae. Try to prevent movement of the larvae and air bubbles from being trapped beneath the coverslip. Gently press on the coverslip with a pencil eraser to spread mouthparts and extrude air. Identify the larvae using a compound microscope. After identification, place a bead of Sally Hanson Hard as Nails around the edge of the cover slip to render the mount permanent. Detailed procedures for the mounting and identification of Chrionomid larvae may be found in EPA (1990).

Store all slide mounted Chironomid larvae in a slide storage box with the corresponding box of subsamples from the same sampling year.

Chironomid pupae are identified to genus (if possible) without subsampling.

3.2.1.2 Mounting Oligochaeta

Place Oligochaetes in a drop of Synasol on a microscope slide. Place several drops of mounting medium (CMCP 10/CMCP 9AF) over the organisms. Carefully place a cover slip over the worms and gently press with a pencil eraser to remove bubbles. Place the slide in a drying oven on low heat for 5 to 10 minutes or until tissues clear.

Place counts of all organisms in the subsample on the Bench Sheet. Include comments on sample condition, etc. in the Comments section of the Bench Sheet. All identified non-Chironomid and non-Oligochaete organisms are placed into a glass snap cap vial and stored in numerical (Log Number) order.

3.2.2 Volunteer Collected Samples (Stream Waders Program)

Identify organisms to family (if possible) except the taxa noted above. Use the Bench Sheet as described above at the family (or higher) level.

4.0 Quality Assurance/Quality Control

4.1 Repeated Subsampling

Using sequential Log Numbers, every 20th sample (if two buckets were required at a site, they should be treated as a single unit) is randomly chosen for re-subsampling and identification according to the following procedure:

A. subsample and identify the sample as usual EXCEPT - identify Chrionomids to Subfamily or Tribe (do not slide mount the larvae) and Oligochaetes to class.

B. return the once-identified organisms to the original sample bucket containing the sortate and preservative, and re-subsample.

C. identify the second subsample according to standard procedures (i.e., slide mount Chironomid larvae and Oligochaetes and identify them to genus and family, respectively, if possible).

D. QC comparisons are made on the two taxa lists and benthic Index of Biotic Integrity (BIBI; see Stribling et al. 1998) values generated from the two subsamples (of the same sample). Differences of less than 1 BIBI value are acceptable.

4.2 Taxonomy

Questionable identifications are verified by consulting other DNR benthic taxonomists, regional experts, and regional keys for certain taxonomic groups.

5.0 Sample Storage and Disposal.

All subsamples are archived indefinitely in 80ml snap top vials containing Synasol. Pencil-written labels include Site ID, sample date, and log number. Vials are stored in cardboard boxes separated and labeled according to sampling year.

Sample sortate is kept in the original 86 ounce plastic bucket for 5 years (e.g., sortate from samples collected during spring 2000 are kept until spring 2005).

Sortate is discarded by pouring the material in the 86 ounce bucket through a #30 sieve over a sink, flushing the Synasol down the drain, and discarding the sample material in an appropriate trash receptacle. Once sortate is discarded, sample buckets are washed and old labels are removed to prepare them for reuse, if possible.

6.0 Literature Cited

Boward, D. M. 2000. Maryland Stream Waders. Volunteer Stream Sampling Manual. Maryland Department of Natural Resources. Monitoring and Non-tidal Assessment Division. Annapolis, Maryland.

EPA 1990. Macroinvertebrate field and laboratory methods for evaluating the biological integrity of surface waters. U.S. Environmental Protection Agency. EPA/600/4-90/030. Cincinnati, Ohio.

Kazyak, P. R. 2000. Maryland Biological Stream Survey Sampling Manual. Maryland Department of Natural Resources. Monitoring and Non-tidal Assessment Division. Annapolis, Maryland.

Stribling, J. B., B. K. Jessup, J. S. White, D. M. Boward, and M. K. Hurd. 1998. Development of a Benthic Index of Biotic Integrity for Maryland Streams. Report Number CBWP-EA-98-3. Prepared for Maryland Department of Natural Resources by Tetra Tech, Inc. Owings Mills, Maryland.

White, J. 1999. Ecological Application Data System (EDAS): A User's Manual. Tetra Tech, Inc. Owings Mills, Maryland.

Appendix A

Contact Information

Benthic sorting and taxonomy

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Benthic macroinvertebrate data management and use in stream assessments

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Ecological Data Application System (EDAS; MBSS modification)

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Appendix B

Maryland Department of Natural Resources
Monitoring and Non-Tidal Assessment Division
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Annapolis, MD 21401

MBSS Benthic Macroinvertebrate Sample Chain-of-Custody Sheet

Site ID	Collector (print)	Collection Date (DD/MM/YY)	Date Delivered to Field Office (DD/MM/YY)	Relinquished By (print)	Received by (print)	Field Office Log Number

Comments

Appendix B (continued)

Guidance for 1997 MBSS Benthic Macroinvertebrate Sample Chain-of-Custody Sheet

General

This sheet provides a means of tracking the transfer of benthic macroinvertebrate samples between field collecting crews and DNR field office personnel responsible for processing the samples. If multiple sample buckets are delivered for a single site, enter each buckets on a separate row. If entries are repeated down a row, it is not necessary to enter the information in each cell. Simply use an arrow or quote marks to indicate the information is repeated down the row. Please write as legibly as possible following the guidelines below. The entry of a printed name indicates responsibility of the individual for relinquishing or receiving each sample.

1. ***Site ID*** Enter the site ID just as it appears on the field data form.

2. ***Collector (print)*** Print the name of the person who collected the benthic sample.

3. ***Collection Date*** Enter the date the sample was collected (using DD/MM/YY format) just as it appears on the field data form.

4. ***Date Delivered to Field Office*** Enter the date the sample was delivered to the field office using DD/MM/YY format.

5. ***Relinquished By (print)*** Enter the printed name of the person relinquishing the sample to the appropriate field office staff member.

7. ***Received By (print)*** Enter the printed name of the person receiving the sample at the field office.

8. ***Field Office Log-In Number*** (Done by field office personnel) Enter the Benthic Sample Log-in number.

9. ***Comments*** Place any pertinent comments regarding the delivered samples, including unusual circumstances, here. Examples include “label for sample from site X fell off - see label in bucket” or “some of sample for site Y spilled while in transport”.

Appendix C
Supplies and Equipment

Sample bucket

86 ounce white plastic bucket (stock number T60785B; 2000 pricing - $649.01 per 1,000)) with snap-on lid (stock number L607; 2000 pricing - $194.74 per 1,000)).

Vendor:

Berry Plastics Corp.
101 Oakley Street
Evansville, Indiana 47706
Phone: 812-424-2904
Fax: 812-424-0128

Note: There is a local (Baltimore area) vendor for this product:

Alternate vendor:

Venture Packaging and Distribution Co. Inc.
6930-A San Tomas Rd.
Elkridge, Maryland 21075-6215
Phone: 410-579-8890
Fax: 410-579-8897

Synasol Preservative (95% denatured ethanol)

55 gallon drum; 2000 price = $243

Vendor:

Maryland Chemical Co., Inc.
1551 Russel Street
Baltimore, Maryland 21230-2090
Phone: 410-752-1800
Fax: 410-433-7891

Subsampling Tray

100cm X 25cm plastic tray with 4" high walls; 100 5cm X 5cm black square grids drawn on the tray bottom. Trays used by DNR were constructed by DNR staff.

Laboratory Sieve

12" diameter white Nalgene sieve; U.S. #30 (595micron mesh); Catalog Number 4230.

Vendor:

Nalgene Labware
75 Panorama Creek Drive
P.O. Box 20365
Rochester, NY 14602-0365

Subsample storage vial

80 ml snap cap vial; catalog number 03-335-10B; 2000 price is $61.09 per case of 72

Vendor:

Fisher Scientific
711 Forbes Avenue
Pittsburgh, Pennsylvania 15219-4785
Phone toll free 1-800-766-7000

Mounting Medium for Chironomidae and Oligochaeta

The two products used are CMCP-9AF and CMCP-10. The mixture used by DNR staff for mounting Chironomidae and Oligochaeta is 2/3 CMCP-9AF and 1/3 CMCP-10.

Vendor:

Masters Company, Inc.
980 Lively Boulevard
Wood Dale, Illinois 60191
Phone 630.238.9292

Appendix D
Portion of the MBSS Benthic Laboratory Bench Sheet

MBSS Benthic Macroinvertebrate
Laboratory Bench Sheet

Office Use Only			
E1D___	Init___	E2D___	Init___

Site: _____-_____-_____-_____ Collection Date _____ Date IDed _____
Log No. _____ Subsampler _____ Taxonomist _____
Collector _____ No. of Grids _____

NEMATOMORPHA		**GAMMARIDAE**		**ODONATA**		**TAENIOPTERYGIDAE**	
GORDIIDAE		*Gammarus sp.*		**AESHNIDAE**		*Oemopteryx sp.*	
				Basiaeschna sp.		*Strophopteryx sp.*	
TURBELLARIA		**TALITRIDAE**		*Boyeria sp.*		*Taeniopteryx sp.*	
Cura sp.		Hyalella sp.					
Dugesia sp.				**CALOPTERYGIDAE**		**HEMIPTERA**	
		PALEMONIDAE		*Calopteryx sp.*		**BELOSTOMATIDAE**	
OLIGOCHAETA		*Palaemonetes sp.*				*Belostoma sp.*	
ENCHYTRAEIDAE				**COENAGRIONIDAE**			
		ISOPODA		*Argia sp.*		**CORIXIDAE**	
LUMBRICULIDAE		**ASELLIDAE**		*Enallagma sp.*		*Palmacorixia sp.*	
		Caecidotea sp.				*Trichocorixa sp.*	
NAIDIDAE		*Lirceus sp.*		**CORDULAGASTRIDAE**			
				Cordulagaster sp.		**GERRIDAE**	
TUBIFICIDAE		**CAMBARIDAE**		**CORDULIIDAE**		*Gerris sp.*	
Limnodrilus sp.		*Cambarus sp.*		*Macromia sp.*		*Trepobates sp.*	
Spirosperma sp.		*Orconectes sp.*		*Somatochlora sp.*		*Limnoporus sp.*	
		Procambarus sp.				*Metrobates sp.*	
HIRUDINEA				**GOMPHIDAE**			
ERPOBDELLIDAE		**INSECTA**		*Arigomphus sp.*		**NOTONECTIDAE**	
		COLLEMBOLA		*Gomphus sp.*		*Bueno sp.*	
PISCICOLIDAE		**ISOTOMIDAE**		*Lanthus sp.*		*Notonecta sp.*	
Piscicola sp.		*Isotomurus sp.*		*Progomphus sp.*			
				Stylogomphus sp.		**VELLIDAE**	
GASTROPODA		**EPHEMEROPTERA**		*Hagenius sp.*		*Microvelia sp.*	
VIVIPARIDAE		**AMELETIDAE**		*Dromogomphus sp.*		*Rhagovelia sp.*	
Campeloma sp.		*Ameletus sp.*					
Viviparus sp.				**LIBELLULIDAE**		**MEGALOPTERA**	
		LEPTOPHLEBIIDAE		*Leucorrhinia sp.*		**CORYDALIDAE**	
		Habrophlebia sp.		*Libellula sp.*		*Chauliodes sp.*	
LYMNAEIDAE		*Leptophlebia sp.*		*Erythemis sp.*		*Corydalus sp.*	
Pseudosuccinea sp.		*Paraleptophlebia sp.*				*Nigronia sp.*	
Radix sp.				**PLECOPTERA**			
Stagnicola sp.		**EPHEMERIDAE**		**CAPNIDAE**		**SIALIDAE**	
		Ephemera sp.		*Allocapnia sp.*		*Sialis sp.*	
				Paracapnia sp.			
PLANORBIDAE						**NEUROPTERA**	
Gyraulus sp.		**EPHEMERELLIDAE**		**CHLOROPERLIDAE**			
Helisoma sp.		*Drunella sp.*		*Alloperla sp.*		**TRICHOPTERA**	
Menetus sp.		*Eurylophella sp.*		*Haploperla sp.*		**BRACHYCENTRIDAE**	
Planorbella sp.		*Ephemerella sp.*		*Sweltsa sp.*		*Brachycentrus sp.*	
Promenetus sp.		*Serratella sp.*				*Micrasema sp.*	
				LEUCTRIDAE			
		HEPTAGENIIDAE		*Leuctra sp.*		**DIPSEUDOPSIDAE**	
PLEUROCERIDAE		*Cinygmula sp.*		*Paraleuctra sp.*		*Phylocentropus sp.*	
Goniobasis sp.		*Epeorus sp.*					
Leptoxis sp.		*Heptagenia sp.*		**NEMOURIDAE**		**GLOSSOSOMATIDAE**	
		Leucrocuta sp.		*Amphinemura sp.*		*Agapetus sp.*	
		Nixe sp.		*Ostrocerca sp.*		*Glossosoma sp.*	
ANCYLIDAE		*Stenacron sp.*		*Prostoia sp.*			
Ferrissia sp.		*Stenonema sp.*		*Shipsa sp.*		**HYDROPSYCHIDAE**	
				Soyedina sp.		*Cheumatopsyche sp.*	

Appendix E

List of Commonly-Used Taxonomic Keys

Merritt, R. W. and K. W. Cummins. 1996. An Introduction to the Aquatic Insects of North America. Third Edition. Kendall/Hunt Publishing Company. Dubuque, Iowa.

Peckarsky, B. L., P. R. Fraissinet, M. A. Penton, and D. J. Conklin, Jr. 1990. Freshwater Macroinvertebrates of Northeastern North America. Comstock Publishing Association. Ithaca, New York.

Pennak, R. W. 1989. Freshwater Invertebrates of the United States. Third Edition. John Wiley and Sons, Inc. New York, New York.

Wiggins, G. B. 1996. Larvae of the North American Caddisfly Genera (Trichoptera). Second Edition. University of Toronto Press. Toronto, Canada.

Appendix F

Overview of Data Entry, Management, and Analysis

Use the Microsoft Access (Office 97 Version) to enter data from the Bench Sheet. This program is an adaptation of the EDAS, details of which can be found in Tetra Tech 1999. Look-up Tables for sites and taxa names are provided in the program. The data entry screen is shown below. All benthic data (including taxon, counts, and site information) are double entered by two individuals.

Once taxonomic counts are entered into the EDAS Program, total organisms per subsample counts are evaluated, along with supplemental environmental (e.g., habitat, water chemistry, land use) data. Those samples with fewer than 60 organisms are evaluated carefully. For these sites, Best Professional Judgement is used to determine if low counts are likely due to sampling error or impairment at the site.

For details on MBSS data management and storage, contact a staff person listed in Appendix A.

Data Entry Screen from EDAS Program